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A hyaluronic acid based lubricious coating for cardiovascular catheters

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ABSTRACT

Hydrophilic lubricious coatings on the catheter surface reduce friction against the blood vessel wall but give rise to iatrogenic complication of particle release, embolization and thin blood vessel blockage leading to pulmonary infarction and stroke. Layer by layer deposited PLL (poly-L-lysine) and HA (hyaluronan) coating fills the urgent need of thinner lubricious coating which does not lead to release of large particles. The 8 layered PLL-HA coating was able to maintain low friction and prevent wear of endothelial glycocalyx layer (EGL). Wear and loss of EGL is a gradual process and occurred while sliding the blood vessel against bare catheter surface or 4 layered coating accompanied by epithelial cells damage and decrease in the number of nucleuses.

1. Introduction

Cardiovascular catheterization is a common medical procedure. Catheters are tubes used in many medical procedures such as catheter angiography, placement of stents, grafts, biopsies, treatment of various diseases, including acute infarction and aneurysms [1–3]. It is estimated that 25% of all hospitalized patients received intravenous infusions and that there are a growing number of outpatients that require frequent catheterization [4]. Catheters are directly contacted the flow biological fluids such as blood or urine during intervention [5]. During catherization the catheter-blood vessel friction develops due to adhesion and deformation [6,7], which induce vasoconstriction and vessel wall injury [8,9].

Many coating solutions are available to reduce the catheter-blood vessel friction [10,11]. These coatings provide advantages such as good wetting, inducement of capillary flow, good biocompatibility, low protein adsorption, and in contact with blood, reduction of the risk of thrombogenesis. For example, phosphonylated low-density polyethylene for vascular applications [12], and hydrophilic highly lubricious coatings [13–18] e.g. hydrophilic poly (vinyl pyrrolidone) (PVP) [19], poly(MPC-co-BMA) phospholipid polymer [18], poly (vinyl alcohol) hydrogel (PVA-H) [20,21], and ComfortCoat® [13] etc. can swell upon hydration. These studies mainly focus on the physical-chemical properties of the structure of coating and study the contact behavior with polymer materials instead of blood vessel tissue.

But these hydrophilic lubricious coatings bring iatrogenic complications [22] due to their use. Distally they can cause inflammation of the radial access site [23] and medially the coating delamination can cause particle release in the blood stream. These hydrophilic coating particles lead to emboli formation [24] causing ischemia at various locations [25], pulmonary infarction [11,26], stroke [26,27] with very high mortality [22]. Thus, the FDA [28], USA has issued a safety alert regarding this iatrogenic risk for hydrophilic lubricious coated cardio-vascular catheters.

The blood vessels affected by embolization are 15–200 μm in diameter [26] indicating that the particles released by the lubricious coatings are in the micrometer size range. Thus there is an urgent need to decrease the thickness of the lubricious coatings which can be effectively applied to the catheter surface. Lower the thickness smaller will be the particles released in the blood vessels. In surface science the use of layer-by-layer (lbl) deposition of polyelectrolyte multilayers is already known to provide nanometer scale coatings, which can be an a feasible solution [29] for this problem. Lbl can host reservoirs and release molecule or particles along with the change of environment [30,31]. As per requirement, the basic properties of the lbl multilayers can be easily modified by changing the molecular weight of polyelectrolytes, their concentration and temperature of deposition etc. [32,33]. As a typical and popular model, biopolymers like anionic HA (hyaluronan) and cationic PLL (polylysine) are used to lbl deposit PLL-HA multilayer coatings, which possess preferable reservoir capacity owning to the changeable thickness from a few nanometers to tens of micrometers [34,35]. PLL-HA coatings has been mainly used for cell culture [36,37],

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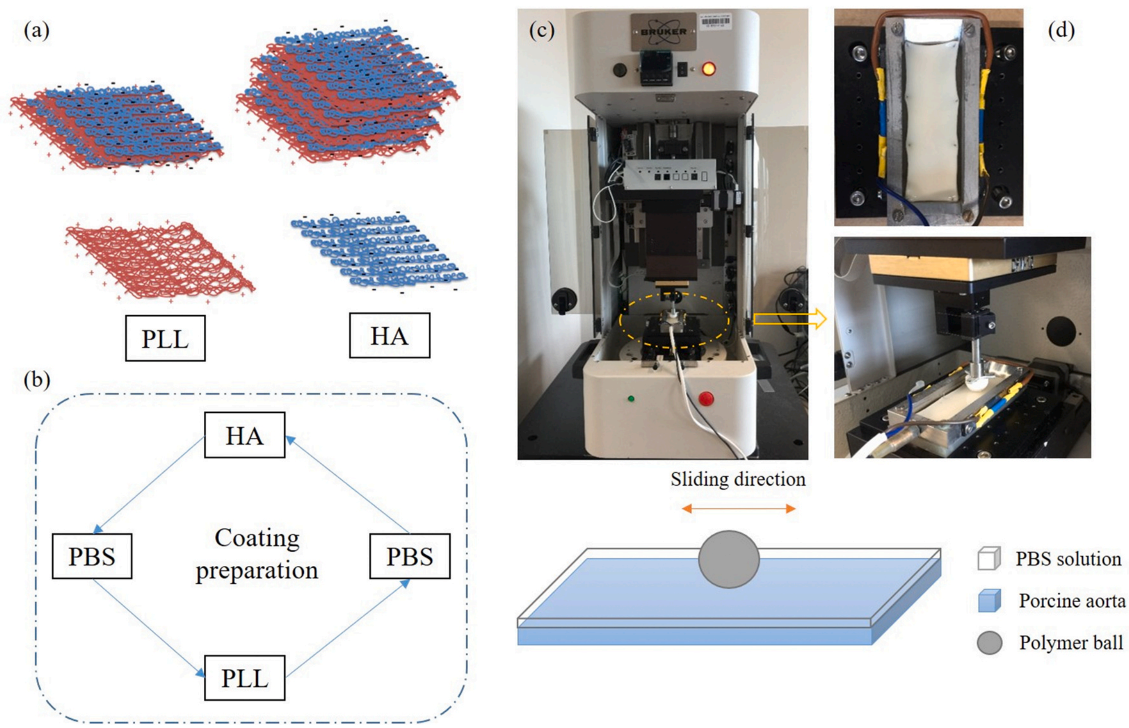


Fig. 1. The preparation process of the PLL-HA layer-by-layer coating and the design of friction experiment: (a) and (b) are the deposition process of different numbers of coating, (c) and (e) for UMT setup, (d) fixture of aorta and (f) for the schematic diagram of contact.

almost no research is available which explores its potential in bio-tribology and bio-lubrication. *In vivo* environment is rich in various biomolecule like proteins, glycoproteins, polysaccharides and lipids, which can absorb on the PLL-HA coatings and further provide desired functionality to the coating. Thus, PLL-HA coatings has great potential in modulating bio-interface properties in vivo.

In present paper, we investigate the lubrication ability of lbl PLL-HA coating for use on the catheter materials surface. We have measured

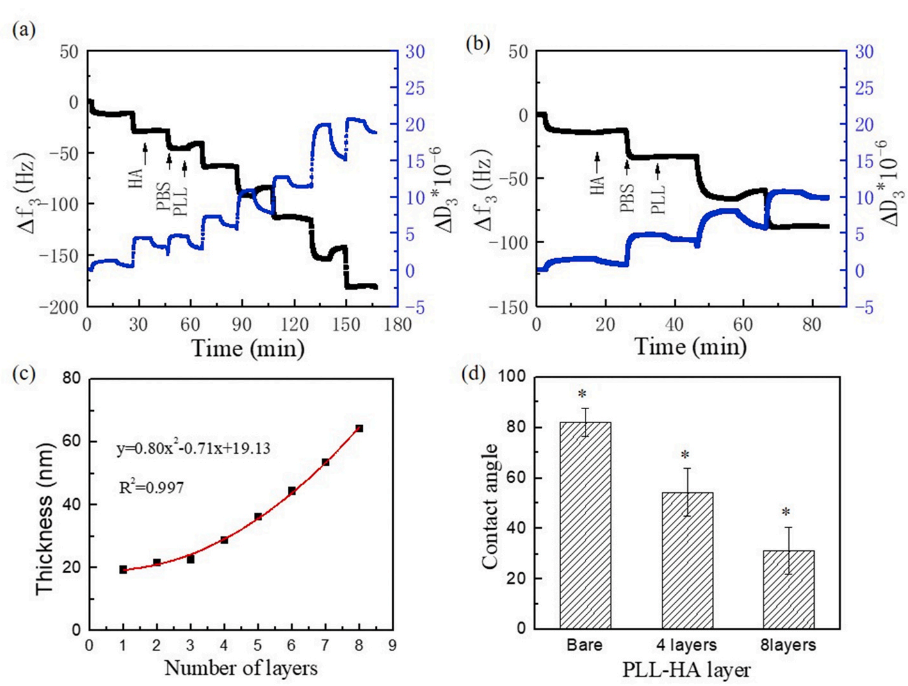
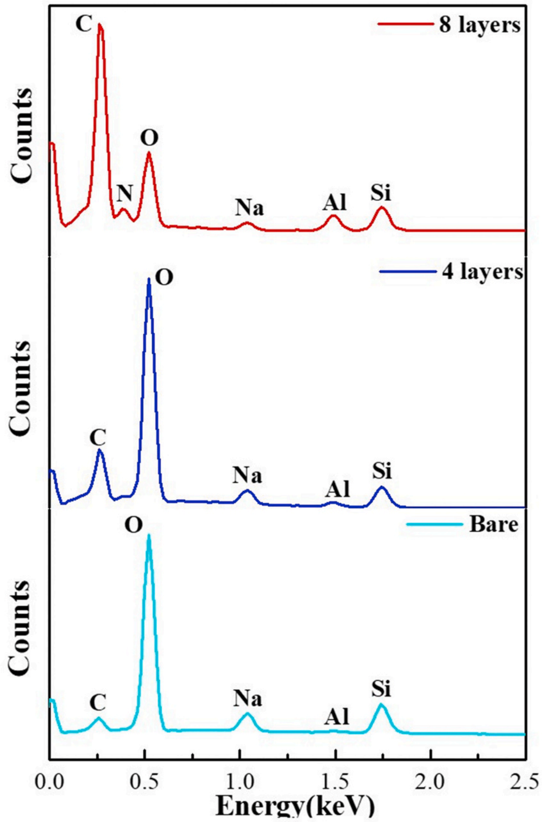


Fig. 2. The QCMD results of the PLL-HA layer-by-layer coating: (a) PLL-HA coating of 8 layers, (b) PLL-HA coating of 4 layers, (c) the thickness of coating and (d) the contact angle of different PLL-HA layer. * means $P < 0.05$.



8 layers:

Element	Weight%	Atomic%
C K	49.90	56.67
N K	14.31	13.94
O K	32.76	27.93
Na K	0.52	0.31
Al K	0.79	0.40
Si K	1.25	0.61

4 layers:

Element	Weight%	Atomic%
C K	26.78	33.35
O K	68.41	63.95
Na K	1.93	1.25
Al K	0.39	0.21
Si K	2.00	1.06

Bare:

Element	Weight%	Atomic%
C K	10.18	13.70
O K	77.93	78.76
Na K	5.72	4.03
Al K	0.23	0.14
Si K	5.72	3.30

Fig. 3. The EDX spectra of bare glass and 4 or 8 layered PLL-HA coatings showing gradual decrease in Si content and increase in C and N content with increase in coating thickness.

kinetics of coating deposition, relevant physico-chemical properties and their friction against blood vessel.

2. Materials and methods

2.1. Sample preparation

There are many cardiovascular disorders which need catheterization. As the thickest artery in the body, the aorta connects to the heart and goes all the way down to the abdomen. Thus most of the catheters would pass through the aorta, making it a very relevant blood vessel to study. So the lumen of the porcine aortas were chosen in this study owning to the similarity in physiological structure and function to the human aorta [38]. The aortas were obtained from the local slaughterhouse (Kroon Vlees, Groningen 9723 TM, Netherlands) under the permission of the Agriculture and Veterinary Authority in Netherland. The weight of each pig was about 90 kg and the age was about 3 months. The length of each intact aorta from the slaughterhouse was 18–22 cm and delivered to the laboratory within 2 h postmortem, and tested within 4 h after extraction so as to avoid dehydration. Firstly, the outside adventitia and the fat layer of aortas were removed with scalpel to ensure the smoothness of samples. Then aortas were cut along the central axis in sample size of 60 mm × 25 mm for friction test, and 5 mm × 5 mm for fluorescent microscopy and SEM (scanning electron microscopy). The wall thickness of the aorta was 1.5–3 mm. The experiment samples were finally washed with phosphate-buffered saline (PBS) solution and then put in refrigerator (4 °C) with PBS solution before the test.

2.2. Lbl deposition of PLL-HA coatings

Polyurethane hemisphere (∅ 16 mm) was chosen as counterpart of aorta to mimic the materials popular for cardiovascular catheter. Sodium hyaluronate (HA; 600 kDa, Kraeber & Cogmbh, Germany) and poly-L-lysine hydrobromide (PLL; 15–30 kDa, CAS 25988-63-0) were supplied by Sigma-Aldrich. PLL-HA layer-by-layer multilayers were deposited on polyurethane hemisphere by first washing with acetone and deionized water and followed by immersing alternatively for 10 min in 1 mg/mL PLL and 1 mg/mL HA with an intermediate rinse with 10 mM PBS solution (pH 7.4). The PLL-HA deposition steps were repeated 4 or 8 times to get two different thickness. The coatings were always terminated with HA as the outermost layer (Fig. 1 a). The thickness of the coating was obtained by fitted a Voigt based model [39]. Namely, by assuming a fluid density of 1000 kg m^{-3}, a fluid viscosity of 1 m Pa s and a layer density of 1100 kg m^{-3} it was possible to estimate the thickness as a function of the number of layers during the build-up of the film [40].

2.3. Friction test

Sliding contact between catheter and blood vessel lumen was mimicked rubbing a polyurethane hemisphere coated with the PLL-HA coating and the lumen of porcine aorta in reciprocating sliding on UMT-3 series multi-specimen Biomedical Micro-Tribometer (UMT-3, Bruker Inc, USA), as shown in Fig. 1. The prepared aorta sample was placed upon the silicon rubber and fixed with pins around the boundary of the aorta (Fig. 1 d). The volume of the PBS solution in bath was 8 mL every time so that the liquid could immerse contact point between the hemisphere and aorta. The temperature of the PBS solution in bath was set at 37°C. The coated hemisphere (counterpart of aorta) was mounted to a suspension system and attached to the load cell. As per the suggestions of the medical practitioners, the normal load was 0.6 N and the sliding speed 6 mm/s. Friction force was measured for 40 min with a sliding distance of 30 mm per cycle using the UMT-3 tribometer at a sampling rate of 20 kHz. Measured friction force was divided by the applied normal force to calculate the coefficient of friction (COF).

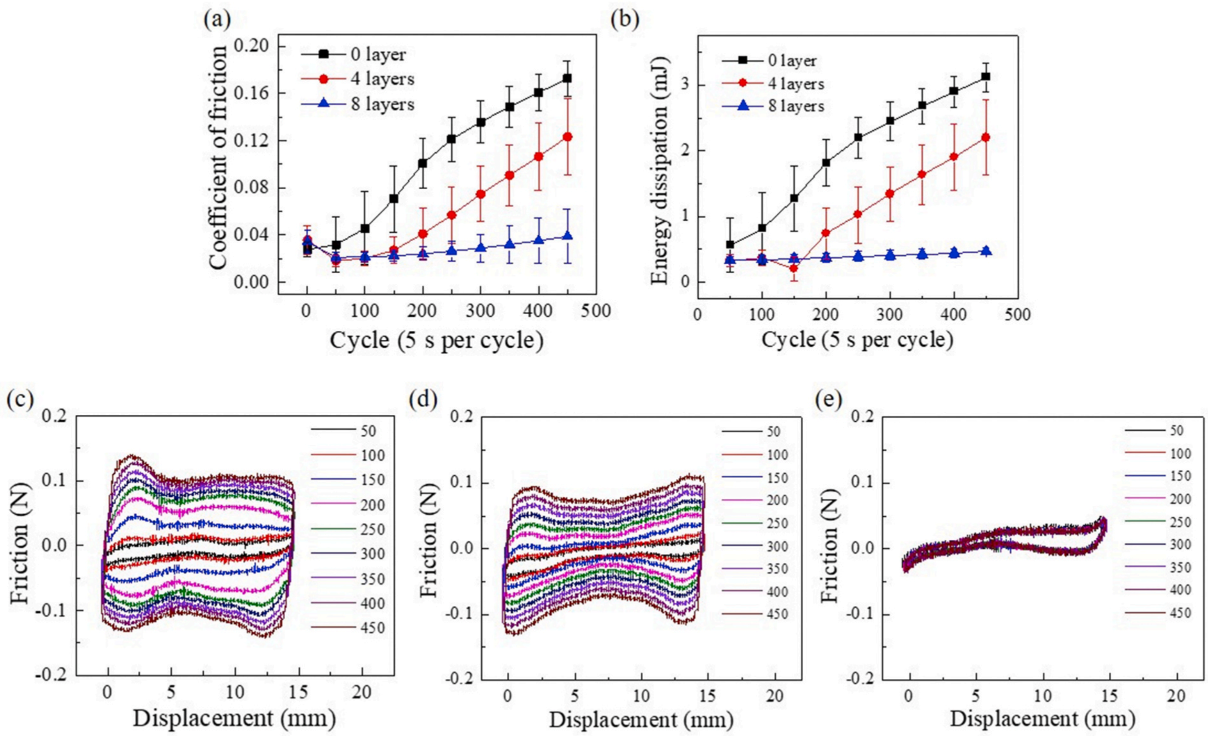


Fig. 4. The coefficient of friction at the ball-aorta interface for different layers of PLL-HA coating: (a) mean value of COF, (b) energy dissipation; The friction-displacement curves at the ball-aorta interface: (c) 0 layer, (d) 4 layers, (e) 8 layers.

Meanwhile, the related energy dissipation can be obtained by integrating the area of friction and displacement [41].

2.4. Glycocalyx visualization and quantification using fluorescent microscopy

The endothelial glycocalyx layer (EGL) on the aorta surface was observed using the Confocal laser scanning microscopy (CLSM, Leica TCS SP2 Leica, Wetzlar, Germany) with an HCX APO L40 × /0.80 WU-V-1 objective. Fluorescent stain Con A (Concanavalin A, Alexa Fluor™ 488 Conjugate from ThermoFisher, Catalog no. C11252) was used to stain the EGL and 6-diamidino-2-phenylindole (DAPI, CAS number 28718-90-3, Sigma-Aldrich) for staining the nucleus of the endothelial cells. An argon ion laser at 488 nm and a green HeNe laser at 543 nm were used to excite Con A and DAPI respectively. The fluorescent signal was collected between 500 and 540 nm for Con A and rendered green while the signal collected between 583 and 688 nm for DAPI was rendered blue. Aorta pieces were submerged in PBS and 1.5% BSA for the same amount of time as negative control. CLSM was used to take fluorescent images, where the excitation laser intensity was kept same consistently for all aorta pieces. Finally, by setting the threshold, the fluorescence intensity was calculated with the ImageJ 1.50b software (Wayne Rasband, National Institutes of Health, USA) [42].

2.5. Surface characterization using scanning electron microscopy (SEM)

The surface of the aorta before and after friction test was visualized with scanning electron microscopy (Nova, FEI, USA). Firstly, the aorta samples were washed with PBS solution, and then fixed in 2.5% glutaraldehyde for 24 h. After the fixation, the samples were washed with PBS thrice (10 min per time). The samples were then immersed in 2% tannin solution twice (15 min per time) and dehydrate using graded ethanol, namely immersed with alcohol with concentrations of 30%, 50%, 70%, 80%, 90%, 95% and 100% (10 min per time). The samples were placed in fume cupboard to dry for 1 h and then coated with gold to make the surface conductive.

2.6. Statistics

The experimental data was presented as the mean value and standard deviation. F-test (analysis of variance) was used to determine the significant difference among different aorta samples under the same test conditions. All the friction tests were repeated three time and the level of statistical significance was set to $P < 0.05$.

3. Results

3.1. Deposition of PLL-HA coating and its characteristics

The Quartz Crystal Microbalance (QCMD) is a high resolution method to measure adsorption at the solid-liquid interface. Fig. 2a and b shows the frequency and dissipation shifts during the layer by layer deposition of 8 and 4 layered PLL-HA coatings. Voigt model fitting to the frequency and dissipation shift data helped us estimate the non-linear increase in PLL-HA layer thickness (Fig. 2c). Four layers of PLL-HA gives rise to 30 nm thick coating whereas 8 layers give rise to 70 nm thick coating.

The contact angle was measured on the custom-built setup. Due to the difficulty in measurement on a sphere, the 4 and 8 layered PLL-HA coating were made on polyurethane (PU) plate. As shown in Fig. 2 (d), bare PU is hydrophobic with a water contact angle of $\sim 80^{O}$ and the angle decreases linearly to 30^{O} for 8 layered PLL-HA coating.

Fig. 3 shows the main composition of glass as silicon dioxide and other oxide, including sodium (Na), aluminum (Al), calcium (Ca) etc. Both PLL and HA are rich in O, C and N, thus as the layers increase from 0 to 8 the C content increase from 14 to 57%. Similarly the nitrogen peak around 0.375 keV clearly is visible for 8 layer and contributes 14% but for 4 layers the N peak is very small and barely visible.

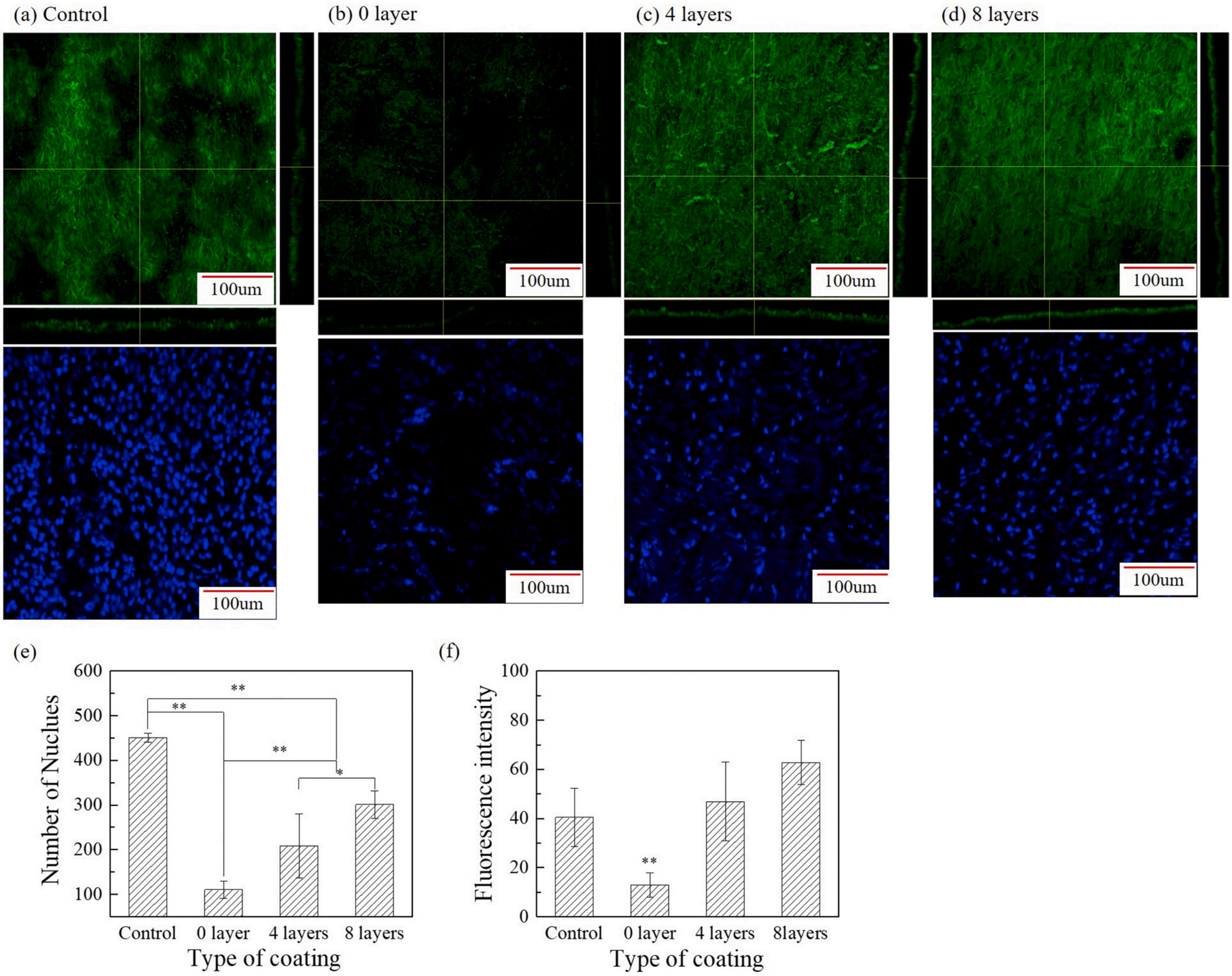


Fig. 5. The fluorescence images of the aorta under different conditions: (a) control, (b) 0 layer, (c) 4 layers, (d) 8 layers (upper images for ConA, nether images for DAPI), (e) the statistical results of number of nucleus and (f) the statistical results of fluorescence intensity. * means $P < 0.05$ and ** means $P < 0.01$.

3.2. The frictional behavior of PLL-HA-aorta interface

During the 40 min of PU sliding against aorta the coefficient of friction (COF) starts very low (~0.03) but almost linearly increases to 0.18 (Fig. 4 a). Similarly, the friction energy dissipation starts at 0.5 mJ and increases to 3 mJ in 40 min (Fig. 4 b). For the 4 layered PLL-HA coating the COF starts around 0.03 and drops to 0.02. The COF remains low for 12.5 min (150 cycles) and then linearly rises to 0.12. Similarly, the frictional energy dissipation remains below 0.2 mJ for 12.5 min and then linearly increased to 0.2 mJ at 40 min. For 8 layered PLL-HA coating the COF and energy remain at 0.02 and 0.5 mJ for the whole period of 40 min i.e. 450 cycles.

3.3. The surface characterization of aorta before and after friction test

The Fig. 5 show the fluorescent intensity of glycocalyx and number of nucleus of the surface of aorta before and after 40 min of sliding against bare and PLL-HA coated PU. Fig. 5 a shows the control aorta surface with nucleuses distributed homogeneously over the surface. All the surface results of the fluorescent intensity of glycocalyx and number of nucleus are repeated three times. After sliding against bare PU the number of nucleuses decrease 4.5 folds (Fig. 5 b, e). Sliding against PLL-HA coated PU causes lower decrease in the number of nucleuses (Fig. 5 c, d and e) with 8 layer performing better than 4 layers. Similarly there is also a difference in the fluorescence intensity between the bare group and other three conditions ($P < 0.05$), as shown in Fig. 5 a, b, c, d and f. Sliding against bare PU causes a big decrease in the presence of EGL, whereas sliding against PLL-HA coated PU preserves the glycocalyx. Sliding against 4 layer shows regions of rolled up glycocalyx.

The SEM (scanning electron microscopy) observation of the aorta surface is qualitative but after 40 min of sliding also shows clear differences after sliding against bare and coated PU (Fig. 6). Fig. 6a–d show pristine aorta surface which is flat and intact under the magnification from 200× to 15000 X, no wear is found on the surface and the texture is very clear. Sliding against bare PU (Fig. 6e–h) shows clear sign of serious damage to the intima of the aorta and rolled up glycocalyx (Fig. 6f and g). In contrast, the sample for 4-layers coating and 8-layers coating show signs of minor damage. But there are still existed wear on the surface as shown in yellow cycles. Under 15000 X magnification (Fig. 6 l and p), the surfaces show a similar texture as control, which means that the PLL-HA coating can protect the aorta surface to different degree. The degree of damage for 4-layers coating is greater than 8-layers coating and the latter is closer to the control group.

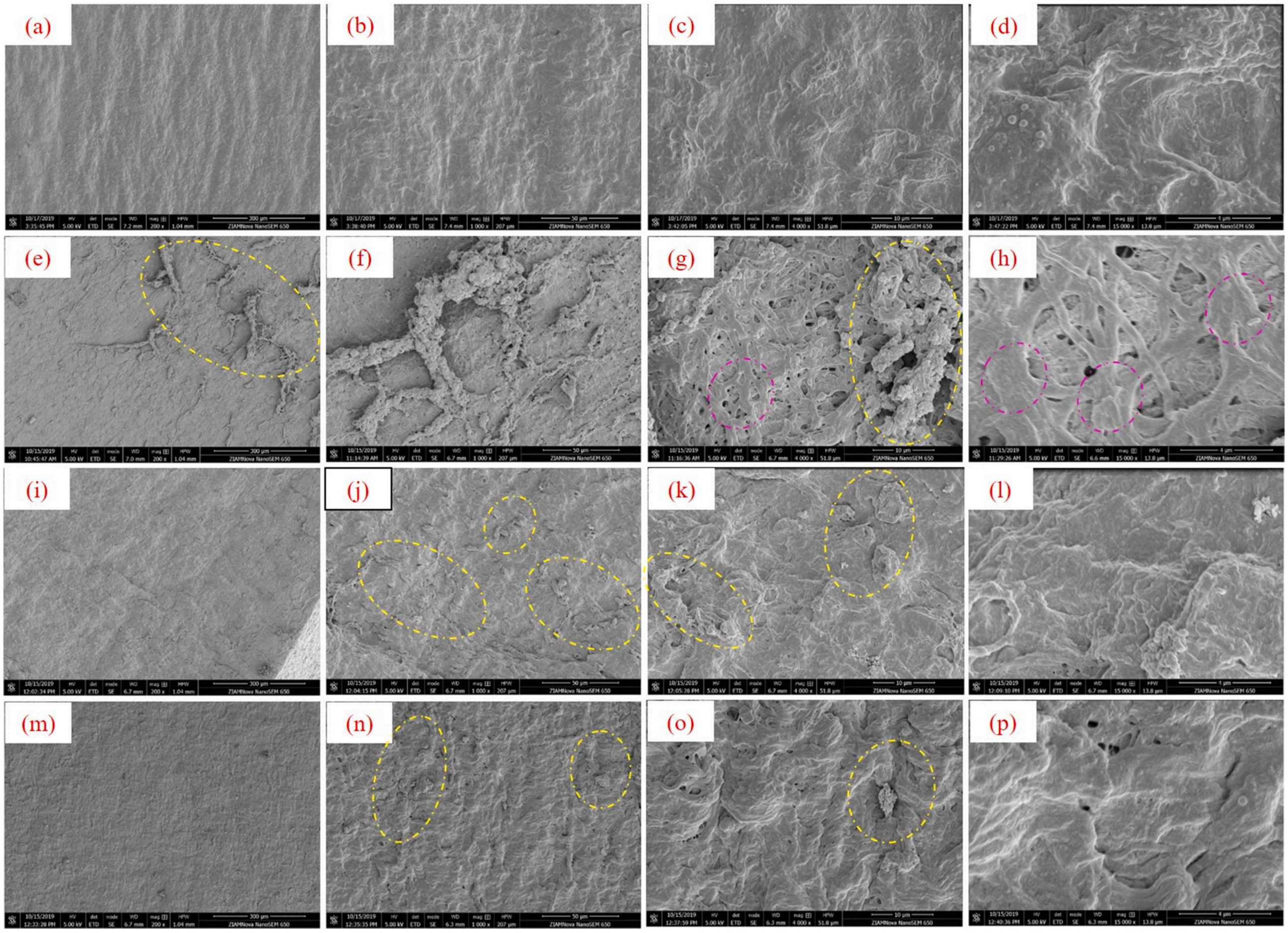


Fig. 6. The scanning electron microscopy images of the aorta under different conditions: (1) control, (2) bare PU, (3) 4 PLL-HA layers, (4) 8 PLL-HA layers. The SEM magnification was 200X, 1000X, 4000X, 15000X from left to right in each row. Yellow circles show sign for wear of tissue, purple circle for scratch. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

We show for the first time that layer by layer deposition of PLL-HA coatings on the PU surface can act as nanometer thick lubricious films for cardio-vascular catheters. Eight layered PLL-HA coatings is preferable because it keeps the COF (Fig. 4 a) and friction energy dissipation (Fig. 4 b) to very low levels for 450 cycles, furthermore it preserves the blood vessel lumen (Figs. 5 and 6) better than the 4 layered PLL-HA.

The level of coefficient of friction at the coating/aorta interface were found to be in the same range as Li et al. [43] and Takashima et al. study [9]. As shown in Fig. 4 (a) and (c) to (e), the COF for all the curves starts very low for different counterparts of aorta, namely different number of PLL-HA coatings. It's mainly due to the lubrication function of the glycocalyx on the surface of aorta. Meanwhile, it can be found the fluorescence intensity of the glycocayx and the number of nucleus both keep a higher level for aorta without wear (Fig. 5). Besides, with the increasing time of friction test, the COF and the related energy dissipation both directly increase while sliding bare PU against aorta. Compared to the unchangeable materials of PU, the only thing which changes is the gycocalyx on the aorta surface as seen in Figs. 5 and 6.

Irrespective of the type of PU surface, at the start of sliding the COF and related frictional energy dissipation are both very low i.e. $\sim$0.03 and 0.5 mJ respectively in Fig. 4. This shows that the EGL which lines the luminal blood vessel surface provides effective lubrication. But as sliding proceeds both the COF and energy increase for bare PU and 4 layer PLL-HA, indicating that the EGL starts losing its structural integrity and is unable to lubricate the catheter-blood vessel interface. The loss of EGL and the wear of the blood vessel endothelial surface i.e. the number of nucleuses are evident from Figs. 5 and 6. Only 8 layer PLL-HA coating is able to preserve the EGL and maintain low COF and frictional energy loss (Figs. 4–6). PBS buffer was used to perform the friction tests, which would keep the glycocalyx in its natural state. The use of serum instead of PBS, in our opinion, will not help preserve the glycocalyx any better that PBS.

Wear followed by loss of EGL and the changes on the endothelial lining of the blood vessel is a gradual process and depends on the robustness of the PLL-HA coating of the PU surface. Bare PU causes an immediate increase in COF and energy dissipation whereas 4 layer PLL-HA maintains low COF and energy for about 150 cycles and only then we see an increase at the same rate as bare PU (Fig. 4 a, b). This indicates that first the 4 layer PLL-HA layer wears off the PU surface and then the EGL gets gradually damaged due to direct sliding against bare PU. The 8 layer PLL-HA seems to be robust enough to lubricate till 450 cycles.

There are rolled up glycocalyx on the luminal blood vessel surface (Fig. 6), especially for the aorta rubbed with bare PU in Fig. 6 f and g. It's a similar phenomenon for the esophagus surface after the friction test [44]. The aorta is made up of intima, media and adventitial from inside to outside [45]. Due to poor lubrication against bare PU, the blood vessel intima is gradually eliminated and the media composed with collagenous fiber and elastin gets exposed on the surface in Fig. 6 h [46]. Meanwhile, accompanied by the sliding process, the epithelial cells are gradually damaged. The endothelial wear seems to be so severe that the cell membranes rupture causing the cytoplasm and nucleus leaked out resulting in the decrease in the number of nucleuses in Fig. 5 (e). These clear signs of EGL wear and superficial necrosis of the endothelial lining required the catheters to be coated with suitable coating, 8 layered PLL-HA coating seems to be a possible solution.

Yet another advantage of this 30–70 nm thick lubricious PLL-HA coating is that even when this coating wears off from the catheter surface we do not expect the particles to be large in size. We expect that the particles released from these coatings in vivo will dramatically reduce the iatrogenic risks of blood vessel embolization.

5. Conclusion

The new design of the PLL-HA coating applied on the cardiovascular catheter was put forward and demonstrated in the paper. Through a series of experiment results and relevant analysis, conclusions can be drew blew:

1. The catheter-blood vessel sliding was mimicked using a new porcine aorta-ball sliding model.
2. There are clear difference for coefficient of friction and frictional energy dissipation between different numbers PLL-HA layers and only 8 layer PLL-HA coating is able to preserve the EGL and maintain low COF and frictional energy loss.
3. Wear followed by loss of EGL and the changes on the endothelial lining of the blood vessel is a gradual process and depends on the robustness of the PLL-HA coating of the PU surface.
4. Accompanied by the sliding against sub optimally coated catheter surface, the epithelial cells are gradually damaged and the number of nucleuses decrease.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Chengxiong Lin: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Funding acquisition. **Hongping Wan:** Resources, Investigation, Writing - review & editing, Funding acquisition. **Hans Jan Kaper:** Methodology, Software, Formal analysis. **Prashant Kumar Sharma:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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